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Neurosteroids: Endogenous Modulators of Seizure Susceptibility

Michael A. Rogawski
National Institutes of Health
Bethesda, Maryland, U.S.A.

Doodipala S. Reddy
North Carolina State University College of Veterinary Medicine
Raleigh, North Carolina, U.S.A.

INTRODUCTION

The term *neurosteroid*, as originally conceived in 1981 by Baulieu, referred to steroids that are synthesized locally within the brain either from cholesterol or steroid hormone precursors (1). More recently, the term has been used in reference to steroids that rapidly alter the excitability of neurons by binding to membrane-bound receptors such as those for inhibitory or excitatory neurotransmitters (2). In common usage, neurosteroid is generally understood to mean an endogenous steroid (whether peripherally synthesized or brain derived) that acts on the nervous system in a non-classical fashion, that is, via cellular actions that do not involve steroid nuclear hormone receptors. The term *neuroactive steroid* encompasses naturally occurring neurosteroids and their synthetic analogs with similar biological properties.

Certain synthetic steroids such as alphaxalone have long been recognized to possess sedative and general anesthetic properties and also to protect against seizures in animals and possibly humans (3–9). It has also been documented that some endogenous steroid hormones, notably progesterone, an ovarian steroid, and deoxycorticosterone (DOC), an adrenal steroid, similarly have sedative and anticonvulsant activities (3,4,8,10,11). These effects occur rapidly and do not correspond with classical notions of steroid hormone action in other target tissues. Indeed, steroids like alphaxalone lack classical hormonal activity but rather act as modulators of neuronal excitability in a fashion similar (although not identical) to barbiturates. This observation became of even greater interest when it was discovered that progesterone and DOC serve as precursors for the endogenous

neurosteroids, respectively, allopregnanolone (5α-pregnane-3α-ol-20-one) and allotetrahydrodeoxycorticosterone (THDOC, 5α-pregnane-3α,21-diol-20-one) that have similar cellular actions to alphaxalone. Like alphaxalone, these neurosteroids do not interact with nuclear hormone receptors, but rather modulate the activity of ligand-gated ion channels, most notably GABA-A receptors.

In this chapter, we review emerging evidence supporting a role for GABA-A receptor–modulating neurosteroids as endogenous regulators of seizure susceptibility and their possible involvement in several conditions of relevance in clinical epilepsy, such as catamenial epilepsy and stress-induced alterations in seizure susceptibility. In addition, we provide an overview of current efforts to develop neurosteroid-based agents for epilepsy therapy. Our focus is mainly on allopregnanolone, but we also touch upon the more recent but still very limited evidence implicating THDOC and 3α-androstanedione (derived from testosterone) in seizure regulation.

BIOSYNTHESIS AND METABOLISM OF NEUROSTEROIDS

GABA-A receptor–modulating neurosteroids are A-ring-reduced metabolites of the hormonal steroids progesterone, DOC, and testosterone. The hormonal steroid precursors of neurosteroids are mainly synthesized in the gonads, adrenal, and fetoplacental unit. The GABA-A receptor active forms are generated by sequential reduction of the parent steroid by 5α-reductase (5α-R) and 3α-hydroxysteroid oxidoreductase (3α-HSOR; also referred to as 3α-hydroxysteroid dehydrogenase) isoenzymes (Fig. 1). These conversion steps largely occur in peripheral tissues that are rich in the two reducing activities. There are two distinct 5α-R isoenzymes that have different tissue distributions. Type I 5α-R is widely distributed throughout the body, and is most abundant in the liver. The type II isoenzyme is primarily expressed in target tissues for androgens, such as the prostate and seminal vesicles. 3α-HSOR activity is also expressed widely. Since neurosteroids are highly lipophilic and can readily cross the blood-brain barrier, neurosteroids synthesized in peripheral tissues accumulate in the brain and can influence brain function (12,13).

In addition to synthesis in peripheral tissues, there is evidence that the neurosteroid biosynthetic enzymes 5α-R and 3α-HSOR are present in brain (14–17). Thus, the steroids can be formed from their parent hormonal steroids directly in the target organ (18,19). Like the neurosteroids, the parent hormones readily enter the brain so that pools of peripherally synthesized precursors are readily available for local neurosteroid biosynthesis. In humans, mRNA for the type I isoenzyme has been demonstrated in neocortex and subcortical white matter as well as in hippocampal tissue specimens obtained from patients with chronic temporal lobe epilepsy (20). The expression levels are about 100-fold lower than in human liver tissue. 5α-R type II mRNA has not been detected in human brain. Similarly, the type I isoenzyme is the predominant form in rat brain, although the type II form is transiently expressed during late fetal and early postnatal life (21) and can be induced in the male brain by testosterone and by progesterone in the female brain (22).

Three functional 3α-HSOR isoenzymes have been characterized. Although the type II isoenzyme is thought to be the main form active in the biosynthesis of neurosteroids (23), a variety of genes that encode proteins related to 3α-HSOR isoenzymes exist and numerous other steroid-reducing enzymes are also capable of catalyzing the formation of neuroactive tetrahydrosteroids from their 5α-dihydro

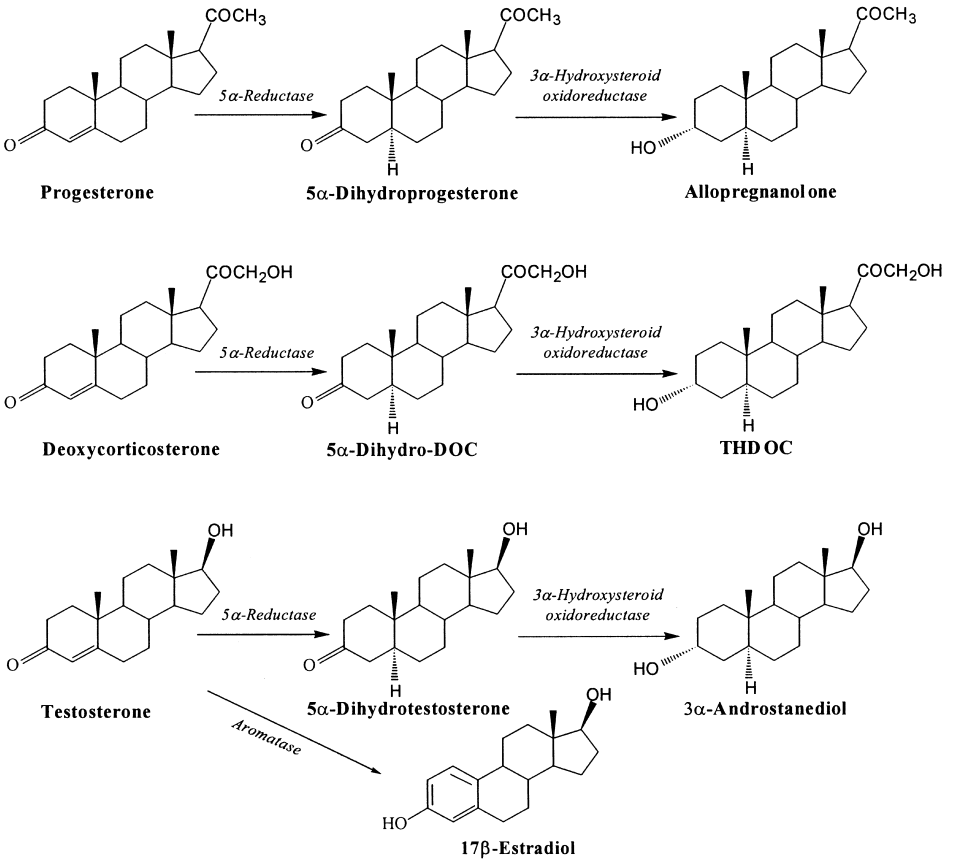

Figure 1. Biosynthetic pathways for the endogenous neurosteroids allopregnanolone allotetrahydrodeoxycorticosterone (THDOC) and 3α-androstanediol.

intermediates. Therefore, the second step in neurosteroid biosynthesis from steroid hormone precursors is likely to be affected by many redundant enzymes. 3α-HSOR activity is found in the pituitary, hypothalamus, and midbrain, and also in limbic structures including the amygdala and hippocampus. Overall, 3α-HSOR activity far exceeds that of the 5α-reductases, so that 5α-reduction is the rate-limiting step in the biosynthesis of neurosteroids (24). 5α-Reductase activity has been identified in both neurons and glial cells (17,25); the cellular localization of the various 3α-HSOR forms has not been determined.

In addition to serving as a site for the conversion of steroid hormones to neurosteroids, there is good evidence that the brain is a steroidogenic organ itself that can synthesize steroid hormones, including progesterone, de novo via classical steroid biosynthetic pathways (26–29). Brain astrocytes and neurons express cytochrome P450 cholesterol side-chain cleavage enzyme ($P450_{SCC}$), which converts cholesterol to pregnenolone, an intermediate necessary for the synthesis of all hormonal steroids (30). Moreover, 3β-hydroxysteroid dehydrogenase, the enzyme required in the further conversion of pregnenolone to progesterone, has been demonstrated in rat brain at the mRNA and protein levels (31). Thus, the enzymes

necessary for the in situ synthesis of progesterone from cholesterol are present in brain. Allopregnanolone persists in the brain after adrenalectomy and gonadectomy or after pharmacological suppression of adrenal and gonadal secretions (32), indicating that progesterone synthesized in situ can be converted to allopregnanolone, as is the case for the peripherally synthesized hormone. Additional enzymes that synthesize critical intermediates such as DOC (21-hydroxylase) are probably also present in brain, as well as enzymes that can synthesize dehydroepiandrosterone and various sex steroids including testosterone (15).

Although the enzymatic steps in local neurosteroid biosynthesis have been well characterized, little is known about the regulation of these pathways. The rate-limiting step in the local biosynthesis of neurosteroids is the reaction catalyzed by $P450_{SCC}$, which is located on the inner mitochondrial membrane. The rate of pregnenolone synthesis is controlled not by $P450_{SCC}$ activity itself but rather the rate at which cholesterol is transported to the inner mitochondrial membrane (33). A receptor that is located on the outer mitochondrial membrane participates in the regulation of intramitochondrial cholesterol transport (34). Diazepam-binding inhibitor (DBI), an endogenous 9-kDa (86–amino acid) peptide, binds to this receptor and stimulates steroidogenesis by facilitating cholesterol transport to the inner mitochondrial membrane (35,36). Mitochondrial DBI receptor is a heterooligomeric complex that has high-affinity recognition sites for the isoquinoline carboxamide PK11195, the imidazopyridine alpidem, the benzodiazepine 4′-chlordiazepam and the 2-arylindoleacetamide FGIN-1-27 (37). These agents have been shown to elicit behavioral effects that are at least partly due to increased neurosteroid synthesis (38,39).

Neurosteroid synthesis can be selectively suppressed by agents that inhibit 5α-R or 3α-HSOR. A variety of 5α-R inhibitors have been developed mainly to inhibit the 5α reduction of testosterone to 5α-dihydrotestosterone as a treatment for benign prostatic hyperplasia and male pattern baldness. The most widely available 5α-R inhibitor is finasteride, which is a synthetic 4-azasteroid and a specific inhibitor of type II 5α-reductase in humans, but a nonspecific inhibitor of both the type I and type II isoenzymes in rodents (24,40,41). 3α-HSOR can be inhibited by various nonsteroidal anti-inflammatory agents, including indomethacin (42). However, because 3α-HSOR activity represents a more diverse group of enzymes than 5α-R and this activity is present in excess (that is, it is not rate limiting like 5α-R), it may be more difficult to suppress neurosteroid biosynthesis with 3α-HSOR inhibitors.

NEUROSTEROIDS ARE POSITIVE ALLOSTERIC MODULATORS OF GABA-A RECEPTORS

Neurosteroids and GABA-A Receptors

The neurosteroids allopregnanolone and THDOC are potent positive allosteric modulators of GABA-A receptors which mediate the bulk of synaptic inhibition in the central nervous system. GABA-A receptors are members of the cysteine-cysteine loop transmitter-gated ion channel family that includes glycine, nicotinic, and $5HT_3$ receptors. They are plasma membrane-bound protein complexes that contain recognition sites for the neurotransmitter agonist GABA and various modulators such as benzodiazepines, barbiturates and neurosteroids (Fig. 2). The central core of the receptor complex serves as an ion channel with high selectivity for Cl^-. Upon

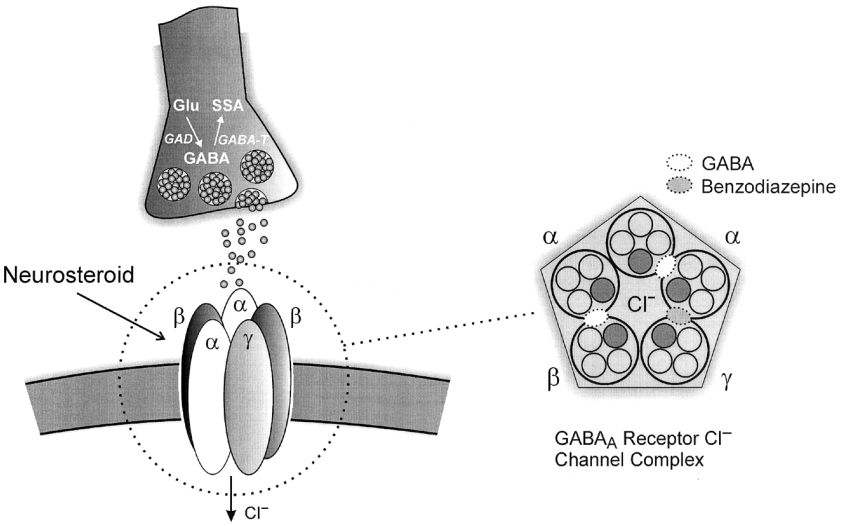

Figure 2. Overview of the inhibitory GABA-ergic synapse—the site of action of neurosteroids. Pathways for GABA biosynthesis and metabolism are schematically illustrated in a presynaptic GABA-ergic nerve terminal. Glutamate (Glu) is converted to GABA by glutamic acid decarboxylase (GAD) and packaged into vesicles. GABA is degraded to succinic semialdehyde (SSA) by 4-aminobutyrate:2-oxoglutarate aminotransferase (GABA-T). The pentameric structure of the GABA-A receptor is illustrated. A typical configuration is believed to be two α subunits, two β subunits, and a γ subunit. Each GABA-A receptor subunit has four transmembrane domains (M1-M4); M2 which forms the pore lining is shown in dark gray. When gated by GABA released from the presynaptic terminal, the GABA receptor permits Cl^- flux. Binding sites for GABA and benzodiazepines are located at subunit interfaces as shown. The binding site for neurosteroids has not yet been delineated but is believed to be distinct from the GABA and benzodiazepine sites.

activation by GABA, the Cl^- channel region of the receptor complex opens, hyperpolarizing the neuron, providing an increase in membrane conductance and effectively shunting the influence of excitatory transmitters such as glutamate (43). GABA-A receptors are believed to be pentameric with the five protein subunits organized like the spokes of a wheel around the ion channel pore. There are seven different classes of subunits, some of which have multiple closely homologous variants ($\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$, $\sigma_{1\text{-}3}$, δ, ε, θ); most GABA-A receptors are believed to be composed of α, β, and γ subunits (44).

The first clues to the way in which some steroids affect neural excitability came from work with the steroid anesthetic alphaxolone (3α-hydroxy-5α-pregnane-11,20-dione) (Fig. 3). Alphaxolone was developed as a short acting general anesthetic based upon Selye's original observations on the anesthetic actions of certain steroid hormones (9,45). In 1980, Scholfield reported that alphaxolone exhibited a barbituratelike action to enhance inhibitory postsynaptic responses in guinea pig olfactory cortex slices, suggesting that alphaxolone may modulate GABA-A receptors (46). This was confirmed in studies demonstrating that alphaxolone enhances GABA-evoked responses and at higher concentrations directly activates GABA-A receptors (47,48). These studies were followed by the demonstration that

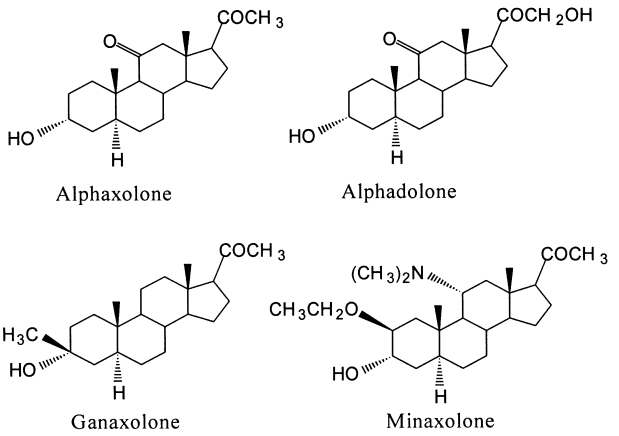

Figure 3. Structures of several synthetic neuroactive steroids. Alphaxalone is often administered as an anesthetic in combination with alphadolone (3α,21-dihydroxy-5α-pregnane-11,20-dione-21-acetate) to improve solubility. Alphadolone has about one-half the potency of alphaxolone.

allopregnanolone and THDOC enhance GABA-A receptor responses in a similar fashion (49).

Molecular Physiology of Neurosteroid Action

There is considerable evidence that neurosteroids bind to GABA-A receptors at a site that is distinct from the recognition sites for GABA, benzodiazepines, and barbiturates (50,51). Behavioral studies with selective antagonists also support the view that neurosteroids do not act through "benzodiazepine receptors" (52–54). Like other positive allosteric modulators of GABA-A receptors, neurosteroids enhance the specific binding of [^{3}H]flunitrazepam, a benzodiazepine receptor agonist, and [^{3}H]muscimol, a specific GABA-site agonist, and inhibit the binding of [^{35}S]*t*-butylbicycloorthobenzoate (TBPS), a cage convulsant and noncompetitive GABA-A receptor antagonist (55–57). Electrophysiological studies have confirmed that neurosteroids act as positive allosteric modulators of GABA-A receptor function (49–50,58–62), and increase the strength of inhibitory postsynaptic potentials (IPSPs) mediated by these receptors (63,64).

Consistent with these studies, neurosteroids potentiate $^{36}Cl^-$ flux stimulated by GABA-A receptor agonists (60,65–67). Neurosteroid enhancement of GABA-A receptor macroscopic currents occurs through increases in both the open frequency and open duration of single GABA-A receptor channels; there is no effect on the single-channel conductance (59,68–70). Kinetic analysis reveals three kinetically distinct open states, and the neurosteroids appear to act primarily by increasing the relative frequency of occurrence of two open states of intermediate and long duration (71). Thus, in the presence of neurosteroids, GABA-A receptors have a greater probability of opening and there is more chloride ion flux so that there is an augmentation of inhibitory GABA-ergic transmission. Studies of near physiological concentrations of THDOC in brain slices have indicated that the effect on inhibitory

synaptic transmission occurs mainly through prolongation of the decay rate of inhibitory postsynaptic currents (IPSCs) rather than an augmentation in their amplitude (72).

GABA-A Receptor Modulation by Endogenous Neurosteroids

Concentrations of allopregnanolone as low as 1 nM are active at GABA-A receptors (73,74). This can be compared to serum concentrations, which in women range from 2 to 4 nM, depending on the phase of the menstrual cycle (75). Brain concentrations are typically higher than in the plasma because of local synthesis (76), and are therefore sufficient to have an ongoing modulatory influence on GABA-A receptor–mediated synaptic inhibition. Moreover, in response to stress, brain levels of allopregnanolone rise rapidly by more than twofold (76). Brain and plasma levels of THDOC rise even more dramatically in response to stress. The low nanomolar concentrations of THDOC present in rat serum after stress are also well within the range of concentrations that enhance GABA-activated chloride currents (11,76). Concentrations near this range have also been shown to enhance GABA-A receptor–mediated synaptic inhibition as assessed by effects on IPSCs (72).

At high concentrations, neurosteroids can directly activate GABA-A receptor channels in the absence of GABA (73). In this respect, neurosteroids resemble barbiturates (77). These direct actions, which are picrotoxin and bicuculline sensitive (11,61), could contribute to the sedative and anticonvulsant effects of exogenously administered neuroactive steroids, but are not likely to be relevant to the actions of endogenous neurosteroids which are not present at sufficiently high concentrations.

GABA-A Receptor Subunit Selectivity of Neurosteroids

GABA-A receptor subunits are differentially expressed both temporally and spatially throughout the brain (44,78). Among the more than 2000 subunit combinations that are theoretically possible, as many as 20–30 distinct forms are likely to exist in the mammalian central nervous system. Most GABA-A receptors are believed to be composed of α, β, and γ subunits with a stoichiometry of 2:2:1. The δ, ε, and θ subunits may replace the γ subunit in some receptor subtypes. The diverse GABA-A receptor forms exhibit a range of physiological and pharmacological properties (79–82). Most forms show neurosteroid modulation, although there are moderate differences depending on the presence and type of α or γ subunit (83). The specific α subunit may influence neurosteroid efficacy, whereas the γ subunit type may affect both the efficacy and potency (EC_{50}) for neurosteroid modulation of GABA-A receptors (173,84–87). In contrast, alterations in the type of β subunit do not appear to affect neurosteroid efficacy or potency (88,89). Substitution of the δ subunit, which is expressed in cerebellum, hippocampus, and thalamus, for γ, results in a small enhancement of neurosteroid efficacy (90). This contradicts the results of Zhu et al. (91), who found that the δ subunit inhibits neurosteroid modulation of GABA-A receptors; the reason for the discrepancy is not known. At higher concentrations, neurosteroids positively modulate GABA-activated Cl^- channels assembled as homopentamers of ρ_1 subunits (92,93) (ρ subunits are mainly expressed in the retina and do not coassemble with GABA-A receptor subunits; the receptors they form have been referred to as GABA-C receptors in recognition of their unique pharmacological properties).

Recently, the use of transgenic animals has begun to unravel the contributions of particular GABA-A receptor subtypes to the specific behavioral actions of the benzodiazepines (94). Whether the various in vivo actions of neurosteroids (e.g., sedative, anxiolytic, and anticonvulsant) can similarly be assigned to specific GABA-A receptor isoforms remains to be determined. In a demonstration of the potential utility of transgenic animals in this respect, Mihalek et al. (95) found that the absence of the δ subunit selectively attenuates behavioral responses to neurosteroids. Furthermore, alphaxolone prolongation of the decay (τ) of pharmacologically isolated miniature GABA-A receptor–mediated synaptic currents (mIPSCs) was much smaller in mice lacking the δ subunit compared with wild littermates (96). This corresponds with the observation noted above that the δ subunit enhances neurosteroid potency.

Since the subunit composition of GABA-A receptors may affect neurosteroid sensitivity, "subunit switching," in which the subunit composition of synaptic GABA-A receptors is altered as a result of hormonal or other factors, is a mechanism by which long-term changes in the responsiveness to neurosteroids can occur (97,98) (see section on neurosteroid withdrawal model of catamenial epilepsy). Inasmuch as classical steroid actions involve regulation of gene transcription, this is a potential mechanism for interplay between the genomic and nongenomic actions of steroids. Moreover, it is becoming apparent that factors such as phosphorylation can alter the activity of GABA-A receptors and may also influence neurosteroid modulation (99,100). Protein kinase activity may be affected by the classic genomic actions of many steroids, thus providing another way in which the genomic actions of steroids can influence neurosteroid sensitivity.

Neurosteroid Structure-Activity Relationships

There are strict structural requirements for neurosteroid modulation of GABA-A receptors. In general, steroids that are potent modulators all have a hydrogen bond-donating 3-hydroxy group in the α configuration on the steroid A ring. In addition, they have a hydrogen bond accepting group (typically a keto moiety) on the D ring at either C20 of the pregnane steroid side chain or C17 of the androstane ring system extending in the opposite β direction (50,60,101). The steroid structure forms a framework that rigidly positions these hydrogen bonding groups in three-dimensional space. The strict requirement for a group in the proper stereochemical orientation at C3 which can engage in hydrogen bonding is further emphasized by the observation that esterification and oxidation of the 3α-hydroxy group greatly reduces activity (102). The orientation of the 5-hydroxy group (which determines whether the A ring is in the coplanar *trans* configuration or in the *cis* boat form) is less critical: β-analogs at this position such as pregnanolone (5β-pregnanone-3α-ol-20-one) are only modestly less active in augmenting GABA receptor–mediated $^{36}Cl^-$ uptake and potentiating GABA-activated Cl^- currents than are the corresponding steroids with the 5α-configuration such as allopregnanolone (61,103). Introduction of a 2β-morpholinyl moiety may confer water solubility for pregnane steroids without loss of GABA-A receptor activity (70). Similarly, substitution of the 3β-hydrogen of allopregnanolone with a methyl group as in ganaxolone (Fig. 3) causes a severalfold reduction in binding affinity to GABA-A receptors (104), but this steroid still produces powerful enhancement of responses at GABA-A receptors.

Neurosteroids with partial agonistic activity have also been reported (105), but the extent to which these are useful as neurosteroid antagonists that could serve as tools for elucidating the physiological roles of endogenous steroids is uncertain. For example, 5β-THDOC has limited efficacy as an allosteric modulator of [^{35}S]TBPS binding, and has been reported to antagonize the action of allopregnanolone and 5α-THDOC at GABA-A receptors in vitro and in vivo (56,84).

Neurosteroids and GABA-A Receptor Plasticity

There is evidence that chronic exposure and withdrawal from neurosteroids elicits changes in the functional properties of GABA-A receptors (106). For example, chronic treatment with allopregnanolone (2–10 μM for 2–5 days) eliminates neurosteroid potentiation of the binding of [^{3}H]flunitrazepam, [^{3}H]Ro15-1788, and other benzodiazepine site ligands in cultured neurons, a process referred to as ''uncoupling'' or the loss of the allosteric interaction between neurosteroids and benzodiazepine recognition sites (107–110). In addition, there are decreases in the binding of other GABA receptor ligands including [^{3}H]GABA and TBPS, referred to as heterologous uncoupling. Along with the alterations in radioligand binding, there are corresponding decreases in the efficacy by which GABA and benzodiazepines stimulate $^{36}Cl^{-}$ influx in neurons that have been chronically exposed to neurosteroids (110). The precise molecular bases for these functional changes in GABA-A receptors and the relevance of these changes for neurosteroid actions in the intact nervous system are not well understood.

In other studies in vivo, withdrawal from chronic allopregnanolone has been reported to upregulate α_4 subunit expression in the hippocampus, leading to alterations in the pharmacology of GABA-A receptors (98,111,112). It has been possible to demonstrate similar changes in α_4 subunit expression in cultured rat cerebellar granule cells upon withdrawal of allopregnanolone (106). Selective increases in α_4 subunit expression are also observed upon withdrawal of benzodiazepines (113). Interestingly, upregulation of the α_4 subunit requires *withdrawal* from chronic exposure to either neuroactive steroids or benzodiazepines; these effects do not occur during the chronic exposure phase. In contrast, in the hypothalamus, there is evidence that chronic neurosteroid exposure (or other hormonal factors) during pregnancy alters the ratio in α_1 and α_2 subunit expression (97,114).

Pregnenolone Sulfate and Dehydroepiandrosterone Sulfate

Although the best-studied action of neurosteroids is positive modulation of GABA-A receptors, some endogenous steroids have inhibitory actions on GABA-A receptors. This mainly occurs with steroids that are sulfated (or alternatively have a hemisuccinate group) at C3 (115). The best-studied examples of such GABA-antagonistic neurosteroids are pregnenolone sulfate (PS) and dehydroepiandrosterone sulfate (DHEAS), which block GABA-A receptors at low micromolar concentrations (116). These steroids act as noncompetitive antagonists of the GABA-A receptor by interacting with a site that is distinct from that at which steroids such as allopregnanolone and THDOC exert their positive modulatory actions (115,117–119). The steroid-negative modulatory action on GABA-A

receptors occurs through a reduction in channel opening frequency, although the precise mechanism of block is not well understood (120,121). PS is present in brain at a relatively high concentration compared with many other neurosteroids (126) and is presumably generated by local steroid sulfotransferases since charged steroid sulfates are unlikely to cross the blood-brain barrier.

In addition to effects on GABA-A receptors, sulfated neurosteroids can also interact with excitatory amino acid receptors including AMPA/kainate and NMDA receptors. The effect on NMDA receptors is of particular note in that NMDA receptor responses are enhanced (122–124). This positive modulatory action occurs in a subunit-dependent fashion with the nature of the NR2 subunit in heteromeric NMDA receptors determining the efficacy of the positive modulatory action of PS (125,126). Thus, NMDA receptors composed of the obligatory NR1 subunit and NR2A or NR2B exhibit PS potentiation whereas those containing NR2C or NR2D are actually inhibited. The mechanism of these effects is not well understood but does not involve the glycine modulatory site on NMDA receptors.

Given their abundance in brain, it seems reasonable that PS and DHEAS could function as endogenous neuromodulators (127–129). However, it has yet to be demonstrated that synaptic concentrations are high enough for modulation of synaptic transmission to occur under normal physiological conditions. In any case, it is interesting that PS and DHEAS can antagonize inhibitory neurotransmission through their actions on GABA-A receptors while also potentiating excitatory transmission by effects on NMDA receptors. The steroids therefore have a dual action that could confer proconvulsant activity and, in fact, the steroids do promote seizures as discussed below.

NEUROSTEROIDS ARE POTENT ANTICONVULSANT AGENTS

Anticonvulsant Profile of Neurosteroids

Like benzodiazepines and barbiturates, GABA-A receptor-positive modulating neurosteroids protect against seizures induced in animals by GABA-A receptor antagonists such as pentylenetetrazol (PTZ), bicuculline, picrotoxin, and methyl-6,7-dimethoxyl-4-ethyl-β-carboline-3-carboxylate (DMCM) (61,130–133). The spectrum of antiseizure activity of some neurosteroids is summarized in Table 1. The neurosteroids also protect against pilocarpine-induced limbic seizures and status epilepticus, and inhibit status epilepticus–like seizure activity induced by prolonged electrical stimulation of the perforant pathway to the hippocampus (133–135). In addition, they protect against the development of kindled seizures, as do other GABA-A receptor–positive modulators (136–138). Some neurosteroids have also been reported to protect against corneal and PTZ-kindled seizures (137,139). At high (and generally behaviorally toxic) doses, neurosteroids also partially protect mice against maximal electroshock (MES)-, kainate-, and NMDA-induced seizures and mortality (140). In addition, neurosteroids are highly efficacious against cocaine, ethanol, diazepam, and neurosteroid withdrawal seizures (66,141,142), indicating a unique broad-spectrum antiseizure activity. Neurosteroids have differing potencies in various seizure models. In mice, the potency ranking is as

Table 1. Pharmacological Profiles of Neuroactive Steroids and Their Precursors in Animal Seizure Models

Steroid	PTZ	Bicuculline	Pilocarpine	Kindling	MES	Kainic acid	NMDA
Precursors							
Progesterone	+	+	+	+	+	0	0
DOC	+	+	+	+	+	0	0
5α-Dihydro-DOC	++			+	+	0	0
Testosterone	?			−		+	
Naturally occurring							
Allopregnanolone	++	++	++	++	+	0	0
Pregnanolone	++	++	++	++	+	0	0
THDOC	++	++	++	++	+	0	0
5α-Dihydrotestosterone	+	+		+	+	0	0
3α-Androstanediol	++	++		+	+	0	0
17β-Estradiol (chronic)				−		−	
Synthetic analogs							
Alphaxolone	++	++		+	+	0	0
Ganaxolone	++	++		+	+	0	0
Minaxolone	++	++					
Sulfated derivatives							
Pregnenolone sulfate	−	−				−	−
DHEA sulfate	−	−				−	−

Compilation of results from studies cited in the text. 5α-Dihydro-DOC serves as a precursor for THDOC and has anticonvulsant activity itself (224).
++, Potent anticonvulsant; +, moderately potent anticonvulsant; −, proconvulsant or convulsant; 0, inactive at nonsedative doses; ?, controversial.

follows (most sensitive to least sensitive): pilocarpine > bicuculline > PTZ > kindling > MES.

Several lines of evidence indicate that the anticonvulsant activity of neurosteroids in these animal models is not related to interactions with traditional steroid hormone receptors that regulate gene transcription. First, the anticonvulsant effects of neurosteroids such as allopregnanolone and THDOC occur rapidly (within minutes). Second, A ring-reduced neurosteroids are not known to directly interact with nuclear steroid hormone receptors. Third, studies in progesterone receptor knockout (PRKO) mice, in which the progesterone receptor has been deleted by gene targeting, conclusively demonstrate that the progesterone receptor is not required for the anticonvulsant activity of neurosteroids (143). Interestingly, the progesterone receptor-deficient animals actually have enhanced sensitivity to the anticonvulsant activity of allopregnanolone and the synthetic neuroactive steroid ganaxolone, and in addition show overall reduced seizure susceptibility; the basis for these alterations is unknown. Although allopregnanolone and THDOC do not themselves appear to interact with intracellular steroid hormone receptors, they may nonetheless indirectly affect progesterone receptors through their oxidized metabolites 5α-dihydroprogesterone or 5α-dihydrodeoxycorticosterone, which can be formed by the reverse action of 3α-HSOR (144).

Anticonvulsant Activity of Progesterone

Progesterone has long been known to have anticonvulsant activity in animal seizure models, and in clinical studies progesterone has been found to reduce the frequency of interictal spikes and lessen the risk of seizures (145–147). The anticonvulsant activity of progesterone in rodent models is similar to allopregnanolone and other GABA-A receptor–modulating neurosteroids, although it is less potent and has a more delayed onset of action (148). The ability of progesterone to protect against seizures, at least in the PTZ test, is eliminated by finasteride, indicating that conversion to 5α-reduced neurosteroid metabolites is required. The conventional (genomic) effects of progesterone in target cells are mediated by progesterone receptors, which are intracellular ligand-activated nuclear transcription factors (149). Progesterone receptors are mainly found in reproductive tissues, but are also expressed in brain. They have a nonuniform distribution with high levels in hypothalamus, and moderate levels in neocortex, hippocampus, and limbic areas (150). As noted in the preceding section, nuclear hormone receptors like the progesterone receptor are not believed to mediate the anticonvulsant activity of neurosteroids. There is also strong evidence from studies in PRKO mice that progesterone receptors are not involved in the acute anticonvulsant activity of progesterone (143). As was the case for allopregnanolone, PRKO mice exhibited enhanced sensitivity to the antiseizure activity of progesterone against PTZ and also kindled seizures. As expected, in the PRKO mice the anticonvulsant effects of progesterone were blocked by finasteride, confirming that conversion to allopregnanolone is required. Although these studies demonstrate that the antiseizure effects of progesterone occur through conversion to allopregnanolone and not via an interaction with progesterone receptors, the possibility that progesterone receptors or other nuclear hormone receptors could play a role in the effects of steroid hormones on seizure susceptibility in some epileptic conditions cannot be excluded (151).

Proconvulsant Effects

As noted previously, the sulfated neurosteroids PS and DHEAS block inhibitory GABA-A receptor responses and also allosterically facilitate excitatory NMDA receptor responses, suggesting that they could be proconvulsant. In fact, acute intracerebroventricular or chronic systemic administration of these steroids reduces the PTZ seizure threshold (152) and intracerebroventricular administration can induce seizures and status epilepticus (153). The seizure-facilitating effects of PS and DHEAS can be blocked by coadministration of allopregnanolone or other related neurosteroids that positively modulate GABA-A receptors, as well as by benzodiazepines and by NMDA receptor antagonists. The overall pharmacological profile suggested that the GABA-A receptor–blocking activity of the sulfated steroids is predominantly responsible for the proconvulsant activity, although the effects on NMDA receptors may also contribute. The possibility that sulphated neurosteroids could have a role in epileptic seizure susceptibility is of interest but has not as yet received experimental support.

It has long been suspected that estrogens can enhance seizure susceptibility and could play a role in seizure exacerbations in women with epilepsy (154). More recently, evidence has accumulated that chronic estrogen treatment has effects on excitatory circuits in the hippocampus that can promote seizure activity (155). In

addition, estradiol has been shown to facilitate kindled seizures, decrease the threshold for electroshock-induced seizures, and increase susceptibility to kainate seizures (156–160). These effects of chronic estrogen do not seem to be due to direct modulatory effects on ion channels comparable to the actions of GABA-A receptor modulating neurosteroids. Rather, estrogens could induce slow changes in brain excitability owing to transcriptional effects. In any case, with either acute or chronic estradiol, we have failed to observe dramatic changes in seizure-susceptibility in mice. As noted below, testosterone has also been claimed to have seizure promoting effects that could occur as a result of its conversion by aromatase to estrogen (138,161).

PHYSIOLOGICAL ROLES AND CLINICAL POTENTIAL OF NEUROSTEROIDS

Although it has been amply demonstrated that neurosteroids are powerful modulators of brain excitability, it has been more difficult to demonstrate a physiological role for neurosteroids in normal or pathological brain function. However, in recent years evidence has steadily accumulated suggesting that neurosteroids are critical biochemical mediators in various clinical conditions, such as premenstrual syndrome, fatigue during pregnancy, and depression, especially in the postpartum period (162–164). Regulation of seizure susceptibility is among the clinical situations in which neurosteroids are likely to play a role; the supporting evidence will be discussed in this section. Recognition of the involvement of neurosteroids in brain disorders may provide clues to novel therapeutic approaches. Indeed, during the last decade, many studies have substantiated the promising therapeutic potential of neurosteroids in a wide variety of neurological and psychiatric conditions including anxiety, depression, learning and memory, and sleep disorders (see reviews, 19,165,166). In this section, we also discuss the potential for neurosteroid-based therapies in epilepsy, focusing on the uses of such agents to treat catamenial and stress-related seizure exacerbations, infantile spasms, and ethanol withdrawal seizures.

Catamenial Epilepsy

A hallmark of epilepsy is the unpredictable occurrence of seizures. However, in many women with epilepsy, seizures do not occur randomly but cluster in association with the menstrual cycle. Based on the review of a vast clinical experience, Newmark and Penry (167) defined catamenial epilepsy as epileptic seizures occurring in women of fertile age exclusively or significantly more often during a 7-day period of the menstrual cycle beginning 3 days prior to menstruation and ending 4 days after its onset. With this criterion as a rough guideline, catamenial epilepsy has been reported to occur in 10–72% of women with epilepsy (168,169). Recently, Herzog et al. (170) proposed an extension of the definition of catamenial epilepsy to include periovulatory and luteal forms. In perimenstrual catamenial epilepsy, the most common clinical type, seizures decrease in the midluteal phase, when serum progesterone levels are high, and increase premenstrually, when progesterone levels fall and there is a decrease in the serum progesterone to estrogen ratio (171,172). As early as 1956, Laidlaw proposed that the premenstrual seizure exacerbations are due to withdrawal of the antiseizure effects of progesterone (173). However, only in

recent years with the recognition that progesterone is converted to allopregnanolone, a powerful anticonvulsant neurosteroid (see above, section on the anticonvulsant activity of progesterone), have the physiological underpinnings of this concept become clear. With this understanding, it is now apparent why perimenstrual catamenial epilepsy may, at least in part, be attributed to withdrawal of the anticonvulsant action of allopregnanolone.

Neurosteroid Withdrawal Model of Catamenial Epilepsy

Despite the increased awareness and understanding of catamenial epilepsy, there are few specific treatment approaches. The dearth of attention to the development of therapies may be due to the lack of an appropriate animal model. Recently, we described a rat model of perimenstrual catamenial epilepsy (142). Through treatment with gonadotropins, a state of chronically elevated serum progesterone and allopregnanolone was induced in immature female rats, referred to as ''pseudopregnancy.'' In pseudopregnancy, secretion of progesterone by the leutinized ovaries occurs in a physiologically appropriate episodic fashion and leads to plasma progesterone levels that are within the physiological range. The magnitude of the increase in serum progesterone is comparable to the six- to eightfold increase that occurs in women during the normal menstrual cycle (75,164). In contrast, the fluctuations in progesterone and allopregnanolone levels in true pregnancy may differ (174). Allopregnanolone was acutely withdrawn by administration of finasteride. On the day following finasteride-induced neurosteroid withdrawal, the animals exhibited increased seizure susceptibility, mimicking the situation in catamenial epilepsy. A similar predisposition to seizures is observed upon abrupt discontinuation of benzodiazepines (175) and ethanol (176), which also have GABA-A receptor–positive modulating properties. Since the fluctuations in neurosteroid levels are similar to those that occur in women during the perimenstrual period, this rat model may replicate the physiological changes that lead to perimenstrual seizure exacerbations and could be useful for the evaluation of therapeutic approaches to the treatment of catamenial epilepsy.

In the development of the rat catamenial epilepsy model, finasteride rather than ovariectomy was used to induce withdrawal from neurosteroids because ovariectomy would be associated with a decrease in estrogens as well as neurosteroids. Ovariectomy would therefore not simulate the reduced ratio of progesterone (and allopregnanolone) to estrogen that is believed to be critical to perimenstrual catamenial epilepsy (177). Nevertheless, ovariectomized pseudopregnant animals did exhibit an increase in seizure susceptibility, indicating that maintained estrogen is not required for enhanced seizure reactivity (178).

The basis for the increased seizure susceptibility following neurosteroid withdrawal in pseudopregnant rats is not well understood, but is unlikely to be due to a reduction in the number of GABA-A receptors. Although high doses of progesterone may downregulate GABA-A receptors as assessed with [^{3}H]muscimol binding (179), pregnant rats appear to have increased brain GABA-A receptor densities by [^{3}H]GABA and [^{3}H]flunitrazepam binding (180). As noted above, Smith et al. (111,112) have proposed that progesterone withdrawal is accompanied by alterations in the expression of GABA-A receptor subunits and a consequent change in GABA-A receptor properties that causes reduced inhibition and an overall

increase in brain excitability. Specifically, these workers reported increased expression of the GABA-A receptor α_4 subunit that was associated with an acceleration in the decay of GABA-A receptor currents in CA1 hippocampal neurons. However, other investigators have failed to observe any change in the expression of the $\alpha_{1\text{-}4}$, $\beta_{1\text{-}3}$, and γ_2S GABA-A receptors subunits in rat cerebral cortex and hippocampus during pregnancy or after delivery, which is associated with a large fall in progesterone and allopregnanolone (174). Therefore, the precise nature of any changes in GABA-A receptors that occur following progesterone withdrawal remains to be characterized.

Neurosteroid Replacement Therapy of Catamenial Epilepsy

Using the rat model of perimenstrual catamenial epilepsy discussed in the preceding section, the pharmacological efficacy of neurosteroids was evaluated with the aim of determining whether neurosteroid ''replacement'' would be an effective approach to protecting against catamenial seizure exacerbations (178). Allopregnanolone and several analogs that act as positive allosteric modulators of GABA-A receptors were tested along with conventional anticonvulsant drugs that are effective in the PTZ model. All neuroactive steroids effectively protected against PTZ-induced seizures. However, there were marked differences between the neuroactive steroids and the other agents in their relative activities in control and withdrawn animals. In all cases, the neuroactive steroids had enhanced anticonvulsant activity in the withdrawn animals, whereas benzodiazepines and valproate exhibited equivalent or reduced anticonvulsant activity. Phenobarbital was similar to the neuroactive steroids in having modestly enhanced activity following neurosteroid withdrawal. These observations suggest that neuroactive steroids may represent a specific treatment approach for perimenstrual catamenial seizure exacerbations due to neurosteroid withdrawal. It is interesting to note that as in the catamenial epilepsy model, the anticonvulsant activity of neurosteroids is also enhanced during withdrawal from chronic ethanol (66,181,234) and diazepam (141).

The molecular mechanisms underlying enhanced neurosteroid anticonvulsant sensitivity following neurosteroid withdrawal are obscure but, like the situation for the overall increase in seizure susceptibility, could be due to changes in the expression of GABA-A receptor subunits associated with withdrawal. There is precedent for reduced benzodiazepine sensitivity following neurosteroid withdrawal and this is likely related to a ''switch'' in GABA-A receptor subunit expression. Thus, the sedative potency of the benzodiazepine lorazepam in animals is reduced after progesterone or neurosteroid withdrawal (182) and attenuated benzodiazepine sensitivity has been observed clinically in patients with the premenstrual syndrome (183), a condition, like catamenial epilepsy, attributed to fluctuations in endogenous neurosteroids. The reduced potency of benzodiazepines has been ascribed to increased expression of the GABA-A receptor α_4 subunit, which confers diazepam and lorazepam insensitivity (111,112). In the rat catamenial epilepsy model, although there was a similar modest reduction in the potency of diazepam following neurosteroid withdrawal, the anticonvulsant activity of bretazenil, a partial benzodiazepine receptor agonist that does act as a positive allosteric modulator of α_4-containing GABA-A receptors, did not show such reduced activity. This is consistent with the view that neurosteroid withdrawal is associated with increased α_4

subunit expression. However, since the α_4 subunit does not modify the sensitivity of GABA-A receptors to neuroactive steroids and barbiturates (80), it seems unlikely that enhanced α_4 expression accounts for the augmented anticonvulsant activity of neuroactive steroids and phenobarbital following neurosteroid withdrawal. Whether changes in the expression of other subunits could lead to enhanced neuroactive steroid sensitivity remains to be determined. It is interesting that neurosteroid sensitivity is enhanced in human subjects being treated with postmenopausal hormone replacement containing progestagens that are not metabolized to neurosteroids (184). It is conceivable that this effect could occur through a similar mechanism as the enhancement that accompanies neurosteroid withdrawal. However, it has recently been shown that the progestagen medroxyprogesterone acetate, which blocks 3α-HSOR, can enhance the local activity of allopregnanolone by preventing its degradation (through oxidation by 3α-HSOR) to the inactive intermediate 5α-dihydroprogesterone (255). This could also explain the observation that medroxyprogesterone, a steroid that does not have GABA-A receptor modulatory activity, is effective in the treatment of catamenial epilepsy (167).

An additional aspect of GABA-A receptor plasticity seen in the catamenial epilepsy model is manifest as reduced antiseizure sensitivity of benzodiazepines in pseudopregnant animals prior to neurosteroid withdrawal (178). These animals have persistently high neurosteroid levels. Since cross-tolerance to benzodiazepines can occur with chronic neurosteroid exposure (see section below), the reduced benzodiazepine sensitivity likely results from such a cross-tolerance mechanism (185). Again, the molecular basis of this phenomenon is not well understood. However, in cultured neurons, chronic exposure to allopregnanolone does markedly reduce the sensitivity of GABA-A receptors to benzodiazepines (108–110).

Ganaxolone in the Treatment of Catamenial Epilepsy

Although natural progesterone therapy benefits some women with catamenial epilepsy (147,186), it may be associated with undesired hormonal side effects. GABA-A receptor–modulating neurosteroids, which are devoid of such hormonal actions, could provide a rational alternative approach to therapy (54). However, certain obstacles prevent the clinical use of endogenously occurring neurosteroids. Importantly, natural neurosteroids such as allopregnanolone have low bioavailability because they are rapidly inactivated and eliminated by glucuronide or sulfate conjugation at the 3α-hydroxyl group. In addition, the 3α-hydroxyl group of allopregnanolone may undergo oxidation to the ketone, restoring activity at steroid hormone receptors (144). Ganaxolone (CCD 1042; 3α-hydroxy-3β-methyl-5α-pregnane-20-one) (Fig. 3), the 3β-methyl analog of allopregnanolone, is an example of a synthetic neurosteroid congener that overcomes these limitations (137). Like allopregnanolone, ganaxolone is a positive allosteric modulator of GABA-A receptors and is an effective anticonvulsant in the PTZ seizure test as well as in other anticonvulsant screening models (132,137 see Table 2). However, ganaxolone is orally active, and adequate blood levels can be maintained in human subjects with BID or TID dosing (187). In addition, although ganaxolone is extensively metabolized, the potentially hormonally active 3-keto derivative is not formed. Preliminary evidence of the efficacy of ganaxolone in the treatment of human epilepsy is presented below, in the section on clinical experience with neuroactive steroids.

Table 2. The Antiseizure Profile of Ganaxolone

Seizure model	Efficacy	References
PTZ	Potent anticonvulsant	132,137,185,188
TBPS	Potent anticonvulsant	137
Bicuculline	Potent anticonvulsant	137
Flourothyl	Potent anticonvulsant	254
Amygdala kindling	Potent anticonvulsant	D.S. Reddy, unpublished data
Corneal kindling	Potent anticonvulsant	137
PTZ kindling	Potent anticonvulsant	139
Cocaine seizures	Moderate anticonvulsant	132
Strychnine	Inactive	137
Aminophylline	Moderate anticonvulsant	137
N-methyl-D-aspartate	Inactive	132
Maximal electroshock	Inactive at nonsedative doses	137
γ-Hydroxybutyrate	Proconvulsant	257

The potential of ganaxolone in the treatment of perimenstrual seizure exacerbations was evaluated in the rat catamenial epilepsy model (188). Like naturally occurring neurosteroids, the anticonvulsant potency of ganaxolone was enhanced in the period following neurosteroid withdrawal, while the potencies of two reference anticonvulsants diazepam and valproate were reduced. There was no corresponding increase in the motor toxicity of ganaxolone, suggesting that the potentiated anticonvulsant activity of ganaxolone results from specific alterations in the brain mechanisms responsible for seizures and is not due to pharmacokinetic factors. Although the protective index of ganaxolone compares unfavorably with that of many conventional anticonvulsant agents (189), following neurosteroid withdrawal there was an increased separation between the doses of ganaxolone-producing seizure protection and motor side effects, suggesting that the drug may be better tolerated during the perimenstrual period of increased seizure frequency. On the basis of measurements of plasma ganaxolone levels, it was possible to estimate the plasma concentrations associated with seizure protection and motor toxicity. In control and neurosteroid withdrawn animals, the threshold plasma concentrations for seizure protection were 200–250 ng/mL and <100 ng/mL, respectively, and the estimated plasma concentrations producing 50% seizure protection were in the range of 450–550 and 200–250 ng/mL. Thus, ganaxolone protects against the PTZ-induced seizures in neurosteroid withdrawn rats at plasma concentrations that are not anticonvulsant in control animals.

Although motor toxicity was not potentiated in the withdrawn animals, it remains to be determined whether the enhanced potency of ganaxolone generalizes to other behavioral effects of neurosteroids, including their sedative-hypnotic, anxiolytic, and cognitive-impairing effects that may be important determinants of side effects in clinical use. If the side-effects profile is acceptable, neuroactive steroids such as ganaxolone could be uniquely suited for the treatment of catamenial seizure exacerbations. In fact, the steroids may specifically overcome the problem of catamenial "breakthrough" seizures during treatment with conventional anticonvulsants

which, if the animal studies are relevant to the clinical situation, may have reduced activity against catamential seizures.

Lack of Tolerance to Neuroactive Steroids

For a neuroactive steroid such as ganaxolone to be of utility in the treatment of catamenial epilepsy, its activity must be maintained with chronic dosing. GABA-A receptor modulating drugs, most notably benzodiazepines such as diazepam, lose activity with chronic dosing due to the development of pharmacodynamic tolerance (190,191). In general, however, anticonvulsant tolerance does not develop to neurosteroids. For example, Kokate et al. (192) demonstrated that the anticonvulsant potency of pregnanolone was not reduced in rats that had received multiple daily doses for up to 2 weeks. In addition, there was no alteration in the pregnanolone plasma concentrations as a result of chronic dosing, demonstrating that there is no induction of metabolism. Because of its longer duration of action, ganaxolone might have a greater liability for tolerance than natural neurosteroids. However, when dosed repeatedly over the course of up to 1 week, tolerance did not develop to the anticonvulsant activity of ganaxolone (185). In addition, tolerance did not develop to the motor toxicity that occurs with higher doses of ganaxolone.

In contrast, there was marked tolerance to diazepam administered according to a similar regimen. Neurosteroids may therefore avoid the problem of tolerance that severely limits the usefulness of anticonvulsants such as benzodiazepines in long-term therapy. Indeed, two recent clinical studies in women with epilepsy (147,186) demonstrated no diminution in the anticonvulsant activity of chronically administered progesterone, which produces anticonvulsant effects via conversion to the neurosteroid allopregnanolone (see above). Similarly, tolerance has not been observed to the anxiolytic and sedative effects of the synthetic neuroactive steroids alphaxolone and 3β-ethenyl-3α-hydroxy-5α-pregnan-20-one (193,194). However, it has been reported that tolerance does occur to the sedative effects of the neuroactive steroid minaxolone (Fig. 3) (195) and the anticonvulsant activity of allopregnanolone when repeatedly administered by intracerebroventricular injections (196).

In the study of Reddy and Rogawski (185) demonstrating lack of tolerance to ganaxolone, it was found that chronic ganaxolone treatment led to cross-tolerance to diazepam. While the molecular basis of this cross-tolerance is not well understood, it could have implications for the clinical use of benzodiaepines. There are fluctuations in endogenous GABA-A receptor-modulating neurosteroids at menarche, during the menstrual cycle, in pregnancy, at menopause, and under stressful circumstances (197,198). In these situations, persistent neurosteroid exposure could lead to reduced benzodiazepine sensitivity and a diminution in clinical efficacy. Reduced benzodiazepine sensitivity has also been associated with *withdrawal* from chronic neurosteroid exposure. Thus, following neurosteroid withdrawal, GABA-A receptor currents have diminished benzodiazepine sensitivity (199), and benzodiazepines exhibit reduced sedative and anticonvulsant actions (111,112,188). Whether neuroactive steroids such as ganaxolone will prove to be superior in clinical situations in which there are fluctuations in neurosteroid levels and reduced benzodiazepine efficacy remains to be determined.

Stress and Seizure Susceptibility

The main focus of attention in studies seeking to understand the importance of neurosteroids in the regulation of seizure susceptibility has been on progesterone-derived allopregnanolone. However, deoxycorticosterone (DOC)-related neurosteroids, which are released in stressful situations, also have central nervous system effects and could affect the propensity for seizures. DOC-related neurosteroids can be considered a component of the hypothalamic-pituitary-adrenal (HPA) axis stress response system. Stress results in the hypothalamic release of corticotropin-releasing hormone (CRH), which liberates adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH is generally understood to act by stimulating cortisol synthesis and release from the adrenal zona fasiculata. However, along with cortisol, ACTH also enhances DOC synthesis in the zona glomerulosa. DOC is a weak mineralocorticoid and serves as a precursor of the major mineralocorticoid aldosterone via 11β-hydroxylation to corticosterone by the enzyme CYP11B1 ($P450_{C11}$). However, DOC synthesis in the zona fasciculata is quantitatively greater than in the zona glomerulosa where its synthesis is under the control of ACTH and its secretion correlates with that of cortisol and not aldosterone (200,201). ACTH causes a relatively greater increase in DOC than in cortisol, and DOC synthesis is not suppressed to the same extent as cortisol by exogenous glucocorticoids (201). Thus, in addition to its well-recognized role as a mineralocorticoid precursor, there is substantial evidence that DOC participates in the HPA axis response to acute stress. The neurosteroid THDOC is synthesized from DOC by the same two sequential A-ring reductions that convert progesterone to allopregnanolone. 5α-R isoenzymes first convert DOC to the intermediate 5α-dihydrodeoxycorticosterone, which is then further reduced by 3α-HSOR to form THDOC (Fig. 1). In contrast to allopregnanolone, which is present in the brain even after adrenalectomy and gonadectomy, THDOC appears to be derived nearly exclusively from adrenal sources (76).

Apparently because of enhanced DOC availability, acute stressors such as swimming, foot shock, or carbon dioxide exposure elicit an increase in THDOC concentrations in plasma and brain (11,76,202–207); Plasma levels of THDOC normally fluctuate between 1 and 3 ng/mL, but increase to 6–10 ng/mL within 10–30 min following acute stress and may reach 15–18 ng/mL in pregnant rats. Proconvulsant GABA-A receptor antagonists such as isoniazid or FG7142 also increase brain levels of THDOC in intact but not in adrenalectomized animals (202,203).

THDOC protects against PTZ-induced seizures and is active in several other chemoconvulsant models as well as against amygdaloid kindled seizures in fully kindled rats (4,11) (Table 1). Seizure protection is also conferred by administration of the precursor DOC. Effects of DOC in the rat PTZ seizure threshold test occur with low doses of DOC that are associated with levels of plasma THDOC comparable to those in stressed animals. Moreover, in rats treated with DOC, there is a good correlation between the degree of seizure protection and the plasma THDOC levels achieved. The protective activity of DOC against PTZ seizures is completely blocked by finasteride, which markedly inhibits the rise in plasma THDOC. Indomethacin, an inhibitor of 3α-HSOR (see section on biosynthesis and metabolism of neurosteroids), also significantly reduced the anticonvulsant activity

of DOC. Taken together, these results indicate that DOC itself is not anticonvulsant and must be activated by A-ring reduction. In fact, in the seizure models, DOC exhibits a relatively delayed onset and more prolonged duration than THDOC, which is compatible with the possibility that DOC is an inactive precursor that must be metabolically activated.

Stress can profoundly influence seizure control in persons with epilepsy (208–210). Moreover, experimental stress has anticonvulsant effects in animals (211,212). However, until recently, the way in which stress affects seizure susceptibility has been poorly understood. Since THDOC exhibits anticonvulsant activity in a variety of animal seizure models (11) (Table 1), it is attractive to speculate that DOC-derived THDOC could play a physiological role in the modulation of seizures.

Recent studies have confirmed that THDOC can participate in the regulation of seizure susceptibility by stress (11). Experimental stress, such as acute swim stress, raises the threshold for the induction of seizures by PTZ and other GABA-A receptor antagonists within ~10 min (211,213). At the time of seizure protection, swim stress is associated with a threefold elevation in plasma THDOC levels (11). Other stressors including footshock produce similar increases in plasma THDOC levels in adult and also in aged animals (76,205). Moreover, the elevation in seizure threshold and the rise in THDOC were eliminated by pretreatment with the 5α-R inhibitor finasteride, consistent with the possibility that the anticonvulsant effect is mediated by 5α-reduced neurosteroids. The stress-induced increase in seizure threshold and THDOC levels were also abolished in adrenalectomized rats (11,76). Overall, the studies strongly implicate THDOC in the protective effect of stress on seizures. The THDOC responsible for this effect could be synthesized in peripheral tissues and then transported by the circulation to the brain or it could be synthesized locally in the brain. Indeed, because of local biosynthesis, brain neurosteroid levels may be substantially higher than plasma levels after stressful events (76). However, based on the results in adrenalectomized animals, it appears that DOC, the precursor for THDOC synthesis, arises exclusively from the adrenal. Allopregnanolone levels are also moderately enhanced by stress, although the effect is not abolished by adrenalectomy. Therefore, while the acute stress-elicited increase in brain allopregnanolone may contribute to the anticonvulsant effects of stress, THDOC is likely to be a more important factor.

Although there are situations where stress or alerting has been shown to reduce epileptiform manifestations, high stress levels and stressful events are more typically associated with more frequent epileptiform EEG spikes and seizures (210,214). Therefore, it is generally accepted that stress triggers seizures (209). Many neural and hormonal factors likely play a role in the regulation of seizure susceptibility during fluctuations in the level of stress. Indeed, studies with neurosteroid synthesis inhibitors in the swim stress model suggested the existence of endogenous proconvulsant factors that could play a role in the precipitation of seizures by stress (11). The extent of seizure susceptibility during stress may therefore represent a balance between anticonvulsant factors, including neurosteroids, and proconvulsant factors. Stress-induced seizures would therefore occur when the balance is shifted to favor the proconvulsant factors, outweighing the anticonvulsant action of endogenous GABA-A receptor–modulating neurosteroids. The proconvulsant mediators have not yet been identified, but could include glucocorticoids (215,216), CRH (217,218), or even ACTH, which may acutely enhance seizure susceptibility possibly

through direct actions on the CNS (214,219,220). Stress is also likely to increase brain levels of pro-convulsant sulfated neurosteroids such as PS and DHEAS, although the extent to which these contribute to the proconvulsant activity of stress has not been defined.

Infantile Spasms

Since the 1950s, ACTH has been known to have beneficial effects in the treatment of infantile spasms and other juvenile epilepsies (221–223). The recognition that ACTH stimulates adrenal DOC synthesis, which leads to enhanced levels of circulating DOC-derived neurosteroids, raises the possibility that the protective activity of ACTH in these epilepsies could, at least in part, be related to neurosteroids (224). Prednisone, a 1,2-reduced steroid that is not biotransformed to neurosteroids, is also well known to have activity in infantile spasms. Therefore, it is unlikely that ACTH effects on neurosteroid synthesis entirely account for its beneficial activity in infantile spasms; stimulation of adrenal glucocorticoids must certainly play a role. However, there is evidence that prednisone is less effective than ACTH (225). The ability of ACTH to stimulate neurosteroid synthesis is one possible explanation for the superiority of ACTH. Treatment with ACTH is still effective against infantile spasms in adrenal-suppressed patients who fail to show a cortisol response to ACTH (226). These studies have been interpreted as indicating that an extra-adrenal mechanism is involved in the anticonvulsant efficacy of ACTH. However, it is notable that DOC synthesis is not suppressed to the same extent as cortisol by exogenous glucocorticoids (201). Although ACTH causes a relatively greater increase in DOC than in cortisol (fourfold for DOC vs. 1.5-fold for cortisol), the glucocorticoid dexamethasone suppresses DOC to a lesser extent than cortisol (41% vs. 95%). Thus, it is conceivable that ACTH may induce an increase in DOC and anticonvulsant neurosteroids even under conditions where the peptide fails to affect cortisol secretion. Given the available evidence, it seems reasonable that neurosteroids could contribute to the anticonvulsant activity of ACTH in infantile spasms and other developmental epilepsies. However, experimental support for this hypothesis is required.

Unfortunately, ACTH has variable and rather undramatic effects on seizure susceptibility in animal models, which has made it difficult to rigorously investigate the hypothesis (227,228). Nevertheless, whether or not the action of ACTH in infantile spasms results to some extent from stimulation of adrenal steroid synthesis leading to increased neurosteroid availability, exogenous neurosteroids—because of their powerful effects on GABA-ergic transmission—would theoretically be expected to have utility as a treatment approach. Indeed, recent clinical trials of ganaxolone support a role for neuroactive steroids in the treatment of infantile spasms (229). Two open-label trials of ganaxolone in infantile spasms have been reported with indications of efficacy in both cases (229). Overall, approximately one-third of 79 patients ages 6 months to 15 years of age with highly refractory infantile spasms showed substantial (>50%) reductions in spasm frequency, with a few subjects becoming spasm-free (229). Detailed information has been provided on 15 children with active refractory infantile spasms who were treated with ganaxolone according to an escalating dosage schedule (230). Many of the children had previously been treated with ACTH or vigabatrin, and all but one were taking conventional antiepileptic drugs throughout the ganaxolone trial. During a 2-month ganaxolone

maintenance period, five of these children experienced >50% decrease in spasm frequency (one became spasm-free), five had a 25–50% reduction, and five did not respond. For the high responders, doses ranged from 18 to 36 mg/kg/d, with serum concentrations in the range of 5.0–51.6 ng/mL (15–155 nM). These ganaxolone concentrations are within the range of those that potentiate recombinant GABA-A receptors expressed in *Xenopus* oocytes ($EC_{50} \sim 100$–200 nM) (137). However, they are substantially lower than the threshold concentrations that are protective in the rat PTZ seizure model (750–950 ng/mL) (185,188), indicating that human infantile spasms could be exquisitely sensitive to neuroactive steroids. In any case, appropriately controlled trials will be necessary to confirm the efficacy of neuroactive steroids in pediatric epilepsies.

Ethanol Withdrawal Seizures

There is extensive evidence demonstrating that ethanol affects endogenous neurosteroid levels. This has led to the speculation that neurosteroids could play a role in the behavioral activity of ethanol and in ethanol tolerance and dependence (231,232). Acute ethanol administration causes substantial increases in plasma and brain allopregnanolone concentrations (233). Moreover, there is a good correlation between the time course of the ethanol-induced increase in allopregnanolone levels and various behavioral effects of ethanol, including its anticonvulsant activity. These effects of ethanol are prevented by finasteride, implicating neurosteroids.

Enhanced seizure susceptibility is an important symptom of ethanol withdrawal in humans that is mimicked in laboratory animals. Devaud et al. (66,181,234) have shown that allopregnanolone and THDOC are five- to 15-fold more potent at enhancing the seizure threshold during the period of potentiated seizure susceptibility following withdrawal from chronic ethanol exposure than they are in control animals. Interestingly, this is opposite to the tolerance and cross-tolerance that develops between ethanol and benzodiazepines (235), but as noted previously is similar to the potentiated activity of neurosteroids seen following neurosteroid withdrawal. The ethanol withdrawal–induced changes in neurosteroid activity are unrelated to the changes in endogenous neurosteroids observed following acute ethanol administration since chronic ethanol consumption is not associated with such elevations in allopregnanolone levels (233). In fact, in human alcoholics, Romeo et al. (236) have found markedly decreased levels of allopregnanolone during early withdrawal from ethanol. Thus, although the mechanisms underlying the enhanced activity of neurosteroids in ethanol withdrawal are obscure, the recent experimental work in animals highlights the potential utility of neuroactive steroids in the treatment of alcohol withdrawal seizures.

Seizure Susceptibility in Men

The incidence of epilepsy is ~15% higher in men than in women at all ages and for most seizure types. Although the underlying mechanisms are poorly understood, androgen deficiency is unusually common among men with epilepsy (237). The most important androgen is testosterone. Unlike progesterone, there are few studies that have investigated the effects of testosterone on neuronal excitability and seizures. Interestingly, however, testosterone and its metabolite 3α-androstanediol exhibit anticonvulsant effects in animal seizure models (161,238,239). On the other hand,

orchidectomized or castrated male animals show significantly higher incidence of seizures to chemoconvulsants (240,241). In addition, male rats are less susceptile to seizures induced by allylglycine (an inhibitor of GABA synthesis) than are female animals (242). Therefore, changes in testosterone levels in men could potentially influence the occurrence of seizures (243).

3α-Androstanediol (5α-androstan-3α,17β-diol, or 17β-dihydroandrosterone; Fig. 1) is structurally very similar to the progesterone metabolite allopregnanolone (50), and it is tempting to speculate that testosterone-derived 3α-androstanediol could play a role in regulating seizure susceptibility. Like allopregnanolone, 3α-androstanediol is synthesized from testosterone by two sequential A-ring reductions. 5α-Reductase isoenzymes first convert testosterone to the intermediate 5α-dihydrotestosterone, which is then further reduced by 3α-HSOR to form 3α-androstanediol (Fig. 1). Testosterone is also converted to 17β-estradiol by aromatase, which, as noted above, may have long-term proconvulsant actions. 3α-Androstanediol is produced de novo by glial cells in the brain (244,245). In addition, the metabolic conversion of testosterone to 3α-androstanediol could also occur in peripheral tissues that express 5α-reductase and 3α-HSOR activities. This raises the possibility that 3α-androstanediol may mediate the effects of testosterone on seizure susceptibility. Although 3α-androstanediol meets the structural requirements for potent GABA-A receptor modulating activity, its effects on GABA-A receptor function have not been widely investigated. There are, however, studies showing that 3α-androstanediol can alter GABA-stimulated chloride flux and muscimol binding (246–248), supporting the view that it could have activity at GABA-A receptors. 3α-Androstanediol can be converted to androsterone by 17β-hydroxysteroid dehydrogenase present in brain and peripheral tissues. Androsterone is also a GABA-A receptor positive modulator with potency about one-tenth that of allopregnanolone (69); there is evidence that it has anticonvulsant properties (256).

Despite testosterone's antiseizure effects in animals (161), however, it has not been reported to have a beneficial effect on seizures in humans (249). One possible explanation is that enzyme-inducing antiepileptic drugs may enhance the conversion of testosterone to estradiol by aromatase, leading to proconvulsant effects. This possibility is supported by the improved seizure control achieved when testosterone is administered with the aromatase inhibitor testolactone or the antiestrogen clomiphene (249,250). In view of these complexities, the role of testosterone-derived neuroactive steroids in the modulation of seizure susceptibility remains elusive.

Clinical Experience with Neuroactive Steroids

In two open-label studies, natural progesterone therapy has been reported to produce dramatic reductions ($\geq$72%) in seizure frequency in women with intractable localization-related epilepsy and catamenial seizure exacerbations (251). In contrast, in published studies of cyclic oral synthetic progestins there was no statistically significant effect on seizure frequency. Since the synthetic progestins are not converted to neurosteroids, it seems likely that the activity of progesterone is related to its ability to form allopregnanolone. Nevertheless, it has been recognized that the open-label trials are open to bias, and a definitive answer regarding the utility of cylic progesterone therapy in catamenial epilepsy awaits the results of an ongoing prospective, randomized, blinded, placebo-controlled study.

Studies with the synthetic allopregnanalone analog ganaxalone also support a role for neurosteroids in epilepsy therapy. A controlled trial in patients with intractable complex partial seizures utilizing an inpatient monotherapy design demonstrated that ganaxolone effectively decreases seizures compared to placebo (252). In other trials, the drug had a favorable safety profile with somnolence, which occurs at higher doses, as the most frequently reported adverse event. As noted previously, open-label data in pediatric patients suggest that ganaxolone may be effective in treating infantile spasms.

In an open-label pilot study, ganaxolone was evaluated for the safety, tolerability, and antiseizure efficacy in two women with catamenial epilepsy (253). Patients received ganaxolone (300 mg/day, PO, BID) starting on the day 21 of the menstrual cycle and continuing through the third full day following the beginning of menstruation. Side effects were mild. During the 4 months of this ganaxolone "pulse" therapy, both patients, who were incompletely controlled with valproate and phenytoin, had a moderate improvement in their catamenial seizures. These promising results warrant further study.

CONCLUSIONS

Although the remarkable GABA-A receptor modulating properties of endogenous neurosteroids have been recognized since 1986 (49), the physiological role of neurosteroids in brain function is still uncertain. Nevertheless, in recent years, evidence has accumulated that neurosteroids could be relevant to several important clinical conditions. Regulation of seizure susceptibility in persons with epilepsy is prominent among these conditions. Hormonal fluctuations in women with catamenial epilepsy, hypogonadism in men, and physiological stress could in part result in alterations in endogenous neurosteroids, which may affect seizure susceptibility.

Synthetic neurosteroids, which lack hormonal properties, have promise in epilepsy therapy. They are particularly likely to be of value in treating hormonally induced fluctuations in seizure susceptibility. In addition, however, they could be useful in a broad range of seizure types, and are of particular promise in infantile spasms.

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